



PHAGE-DEPENDENT SUPERPRODUCTION OF BIOLOGICALLY
ACTIVE PROTEIN AND PEPTIDES

Kordyum, et al.

Appl. No.: 09/929,918 Atty Docket: PHAGE.006A

FGF fr HUMECGFB	TTC	GCC	CTG	ACC	AAG	AAT	CTG	CCT	CCA
haFGF 155	TTT	GCG	TTA	ACG	AAA	AAC	CTT	CCG	CCC
No amino acid	009	011	012	013	015	017	018	019	020
	Phe	Ala	Leu	Thr	Lys	Asn	Leu	Pro	Pro
FGF fr HUMECGFB	AAG	AAG	AAA	CTC	CTC	TGT	AGC	GGG	GGC
haFGF 155	AAA	AAA	AAG	CTT	CTT	TGC	AGT	GGA	GGA
No amino acid	024	025	027	028	029	031	032	034	035
	Lys	Lys	Lys	Leu	Leu	Cys	Ser	Gly	Gly
FGF fr HUMECGFB	AGG	ATC	CTT	CCG	GTG	ACA	AGG	GAC	AGG
haFGF 155	CGA	ATT	CTG	CCA	GTA	ACT	CGC	GAT	CGC
No amino acid	039	040	041	042	046	049	050	051	052
	Arg	Ile	Leu	Pro	Val	Thr	Arg	Asp	Arg
FGF fr HUMECGFB	AGC	CAG	AGT	GCG	GTG	GGG	GTG	ATA	AGT
haFGF 155	TCC	CAA	TCG	GCC	GTT	GGA	GTC	ATC	TCG
No amino acid	053	060	062	063	066	067	069	071	073
	Ser	Gln	Ser	Ala	Val	Gly	Val	Ile	Ser
FGF fr HUMECGFB	ACC	TTG	GAC	TTA	TAC	ACA	CCA	AAT	GAG
haFGF 155	ACG	CTT	GAT	CTG	TAT	ACG	CCT	AAC	GAA
No amino acid	074	080	085	088	089	093	094	095	096
	Thr	Leu	Asp	Leu	Tyr	Thr	Pro	Asn	Glu
FGF fr HUMECGFB	TGT	TTC	CTG	AGG	CTG	GAG	GAG	ACC	TAT
haFGF 155	TGC	TTT	CTA	AGA	CTA	GAA	GAA	ACG	TAC
No amino acid	098	100	101	103	104	105	106	111	112
	Cys	Phe	Leu	Arg	Leu	Glu	Glu	Thr	Tyr
FGF fr HUMECGFB	TCC	AAG	AAG	AAT	GTT	CTC	AAG	AAG	GGG
haFGF 155	TCG	AAA	AAA	AAC	GTA	CTT	AAA	AAA	GGT
No amino acid	114	115	116	121	124	126	127	128	130
	Ser	Lys	Lys	Asn	Val	Leu	Lys	Lys	Gly
FGF fr HUMECGFB	AGC	TGC	AAA	CGC	GGT	CCT	CAG	AAA	GCA
haFGF 155	TCC	TGT	AAG	CGT	GGA	CCA	CAA	AAG	GCT
No amino acid	131	132	133	134	135	136	142	143	144
	Ser	Cys	Lys	Arg	Gly	Pro	Gln	Lys	Ala
FGF fr HUMECGFB	TTT	CTC	CCC	CTG	GTC	TCT	TCT	GAT	
haFGF 155	TTC	CTG	CCA	CTC	GTG	AGC	TCC	GAC	
No amino acid	147	148	149	150	152	153	154	155	
	Phe	Leu	Pro	Leu	Val	Ser	Ser	Asp	

MODIFICATION IN MOLECULE haFGF 155 CODONS.

FGF fr HUMECGFB—THE SEQUENCE FROM GENBANK(at NCBI), haFGF 155
—THE SEQUENCE SYNTHESIZED BY THE INVENTION AUTHORS.

FIG.2



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		BsaBI							

		BsaBI							

		BglII		VspI		BfmI		MbiI	
		-----		-----		-----		-----	
4951		CGCTAGAGGATCGAGATCTCGATCCCGCGAAATTAATACGACTCA		CTATAGGGGAATTGTGAGCGGATAACAATTCCTCTAGAAATAA					
		CGCATCTCCTAGCTCTAGAGCTAGGGCGCTTAATTATGCTGAGT		GATATCCCTTAACACTCGCCTATTGTTAAGGGGAGATCTTTATT					
<hr/>									
+2				MetAsnTyrLysLys		ProLysLeuLeuTyrCysSerAsnGlyGlyHisPheLeuArgIl			
						PstI			

				NdeI		HindIII		BfmI	
				-----		-----		-----	
5041		TTTTGTTTAACTTTAAGAAGGAGATATACATATGAATTACAAAAA		ACCCAAGCTTCTTTACTGCAGTAACGGAGGACACTTCTGCGAAT					
		AAAACAAATTGAAATTCTTCCTCTATATGTATACTTAATGTTTTT		TGGGTTTGAAGAAATGACGTCATTGCCTCTGTGAAGGACGCTTA					
<hr/>									
+2		eLeuProAspGlyThrValAspGlyThrArgAspArgSerAspGln		HisIleGlnLeuGlnLeuSerAlaGluSerValGlyGluValTy					
				PvuI					

		EcoRI		NruI		PvuII		XmaIII	
		-----		-----		-----		-----	
5131		TCTGCCAGATGGCACAGTAGATGGGACTCGCGATCGCTCCGACCA		GCACATTGAGTGCACACTTCGGCCGAAAGCGTTGGAGAGGTCTA					
		AGACGGTCTACCGTGTCTATCTACCCTGAGCGCTAGCGAGGCTGGT		CGTGTAAAGTCGACGTTGAGAGCCGGCTTTTCGAACCTCTCCAGAT					
<hr/>									
+2		rIleLysSerThrGluThrGlyGlnTyrLeuAlaMetAspThrAsp		GlyLeuLeuTyrGlySerGlnThrProAsnGluGluCysLeuPh					
		SalI							

		HindII		RsaI		StyI			
		-----		-----		-----			
		HincII		MlsI		Csp6I			
		-----		-----		-----			
								Mval269I	

5221		TATCAAGTCGACGGAGACTGGCCAGTACCTTGCCATGGACACCGA		TGGGCTTCTGTATGGCTCACAGACGCTAACGAAGAATGCTTGTT					
		ATAGTTCAGCTGCCTCTGACCGGTCTGGAACGGTACCTGTGGCT		ACCCGAAGACATACCGAGTGTCTGCGGATTGCTTCTTACGAACAA					
<hr/>									
+2		eLeuGluArgLeuGluGluAsnHisTyrAsnThrTyrIleSerLys		LysHisAlaGluLysAsnTrpPheValGlyLeuLysLysAsnGl					
				RsaI					

		XbaI		Csp6I				StuI	
		-----		-----				-----	
5311		TCTAGAAAGACTAGAAGAAAACCATTACAACACGTACATATCGAA		AAAACATGCAGAGAAGAACTGGTTTGTAGGCCTTAAAAAAATGG					
		AGATCTTTCTGATCTTCTTTTGGTAATGTTGTGCATGTATAGCTT		TTTTGTACGTCTCTTCTTGACCAACATCCGGAATTTTTTTTACC					
<hr/>									
+2		ySerCysLysArgGlyProArgThrHisTyrGlyGlnLysAlaIle		LeuPheLeuProLeuProValSerSerAsp***					
						SacI			

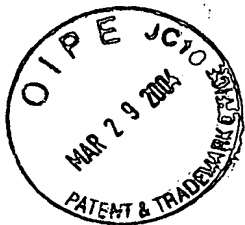
				MlsI				Ec1136II	
				-----				-----	
								BamHIEcoRI	

5401		TTCTGTAAAGCGTGGACCACGGACTCACTATGGCCAAAAGGCTAT		CTTGTTCTGCCACTACCAGTGAGCTCCGACTAAGGATCCGAATT					
		AAGGACATTGCGACCTGGTGCCTGAGTGATACCGGTTTTCCGATA		GAACAAGGACGGTGATGGTCACGAGGCTGATTCTCCTAGGCTTAA					
<hr/>									
		SacI		SalI					
		-----		-----					
		EcoRI		HindII		XmaIII		XhoI	
		-----		-----		-----		-----	
		Ec1136II		HincII		HindIII		NotI	
		-----		-----		-----		-----	
								AvaI	

5491		CGAGCTCCGTCGACAAGCTTGGCGCCGCACTCGAGCACCACCACC		ACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTG					
		GCTCGAGGCAGCTGTTTGAACGCCGGCGTGAGCTCGTGGTGGTGG		TGGTGGTGACTCTAGGCCGACGATTGTTTCGGGCTTCTCCTCGAC					

NUCLEOTIDE AND AMINO ACID SEQUENCE FOR CHEMICALLY
SYNTHESIZED HUMAN A FGF (134 AMINO ACIDS)

FIG. 6



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2971 GGGCGCTGACTTCCGCGTTTCCAGACTTTACGAAACACGGAAACC GAAGACCATTTCATGTTGTTGCTCAGGTCGCAGACGTTTTGCAGCA
CCC GCGACTGAAGGCGCAAAGGTCTGAAATGCTTTGTGCCTTTGG CTCTCGTAAGTACAACAACGAGTCCAGCGCTCGCAAAACGTCGT

BpiI

3061 GCAGTCGCTTCACGTTGCTCGCGTATCGGTGATTTCATTCTGCTA ACCAGTAAGGCAACCCCGCCAGCCTAGCCGGTCTCAACGACAG
CGTCAGCGAAGTGCAAGCGAGCGCATAGCCACTAAGTAAGACGAT TGGTCATTCCGTTGGGGCGGTCCGATCGGCCAGGAGTTGCTGTC

PpuMI
EcoO109I
DraII
AvaII

3151 GAGCAGATCATGCGCACCCGTGGGGCCGCCAGATCTCGATCCCG CGAAATTAATACGACTCACTATAGCGGAATTGTGAGCGGATAACA
CTCGTGCTAGTAGCGGTGGGCACCCCGCGGTCTAGAGCTAGGGC GCTTTAATTATGCTGAGTGATATCCCTTAACACTCGCCTATTGT

BsaBI
NsbI BglII VspI BfmI MbiI

+2 MetAlaGluGlyGluIleThrThrPheThrAlaLeuThrGl
HpaI
HindII
HincII

3241 ATTCCCTCTAGAAATAATTTTGTAACTTTAAGAAGGAGATAT ACATATGGCTGAAGGGGAAATCACCACCTTTACAGCGTTAACGGA
TAAGCGGAGATCTTTATTAAACAAATTGAAATTCTTCTCTATA TGTATACCGACTTCCCTTTAGTGGTGGAATGTGCGCAATTGCCT

XbaI NdeI Eco57I

+2 uLysPheAsnLeuProProGlyAsnTyrLysLysProLysLeuLeu TyrCysSerAsnGlyGlyHisPheLeuArgIleLeuProAspGl
SmaI PstI
AvaI HindIII BfmI EcoRI

3331 GAAATTTAACCTTCCGCCCCGGAATTACAAAAACCAAGCTTCT TTAAGTGCAGTAACGGAGGACACTTCTGCGAATTCTGCCAGATGG
CTTTAAATTGGAAGCGGGCCCTTAATGTTTTTTGGGTTTCAAGA AATGACGTCATTGCCTCTGTGAAGGACGCTTAAGACGGTCTACC

+2 yThrValAspGlyThrArgAspArgSerAspGlnHisIleGlnLeu GlnLeuSerAlaGluSerValGlyGluValTyrIleLysSerTh
Sali
HindII
HincII

PvuI XmaIII
NruI PvuII CfrI

3421 CACAGTAGATGGGACTCGCGATCGCTCCGACCAGCACATTAGCT GCAACTCTCGGCCGAAAGCGTTGGAGAGGTCTATATCAAGTCCGAC
GTGTCATCTACCCTGAGCGCTAGCGAGGCTGGTCGTGTAAGTCGA CGTTGAGAGCCGCTTTCGCAACCTCTCCAGATATAGTTTCAGCTG

+2 rGluThrGlyGlnTyrLeuAlaMetAspThrAspGlyLeuLeuTyr GlySerGlnThrProAsnGluGluCysLeuPheLeuGluArgLe
MlsI RsaI StyI
CfrI Csp6I NcoI AcyI Mva1269I XbaI

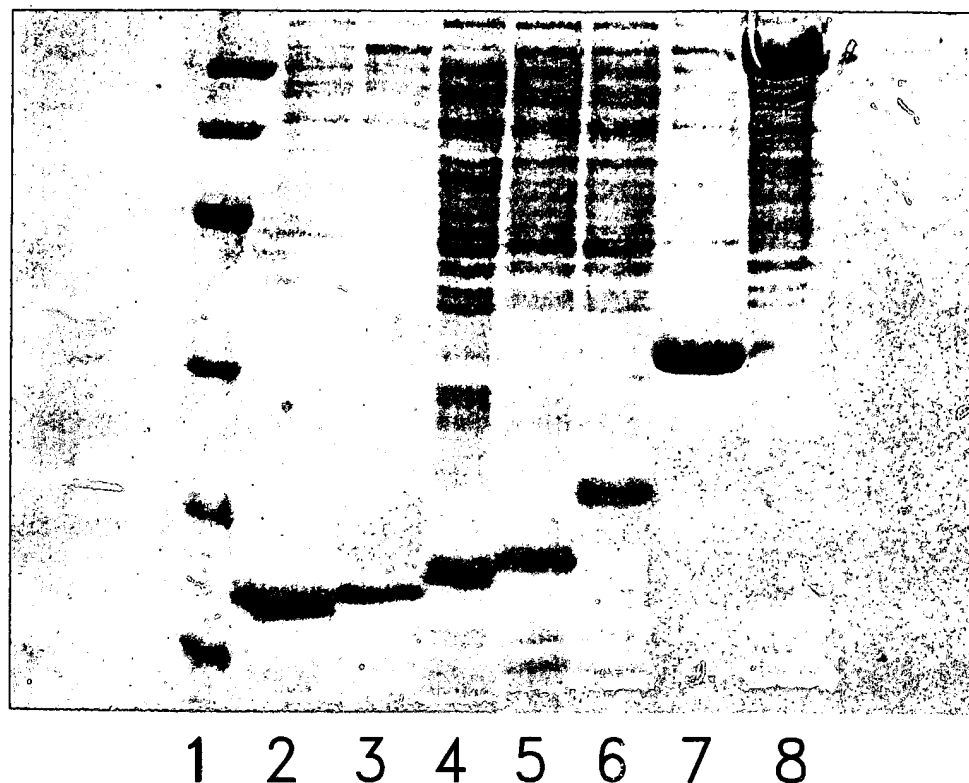
3511 GGAGACTGGCCAGTACCTTGCCATGGACACCGATGGGCTTCTGTA TGGCTCACAGACGCTAACGAAGAATGCTTGTCTAGAAAGACT
CCTCTGACCGGTATGGAACGGTACCTGTGGCTACCCGAAGACAT ACCGAGTGTCTGCGGATTGCTTCTTACGAACAAGATCTTTCTGA
BsmAI

NUCLEOTIDE AND AMINO ACID SEQUENCE FOR CHEMICALLY
SYNTHESIZED INTERFERON α -2B

FIG. 10



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ELECTROPHOREGRAM

1-MOLECULAR WEIGHT KIT (94 000,67 000,43 000,30 000
20 100, 14 4000)

THE CULTURAL MEDIUM, CONTAINING:

2-haFGF 134(40 μ l OF THE CULTURAL MEDIUM)

3-haFGF 140(40 μ l OF THE CULTURAL MEDIUM)

4-IFN α 2B(40 μ l OF THE CULTURAL MEDIUM)

5-haFGF 155(40 μ l OF THE CULTURAL MEDIUM)

6-HGH(40 μ l OF THE CULTURAL MEDIUM)

7-MAP(40 μ l OF THE CULTURAL MEDIUM)

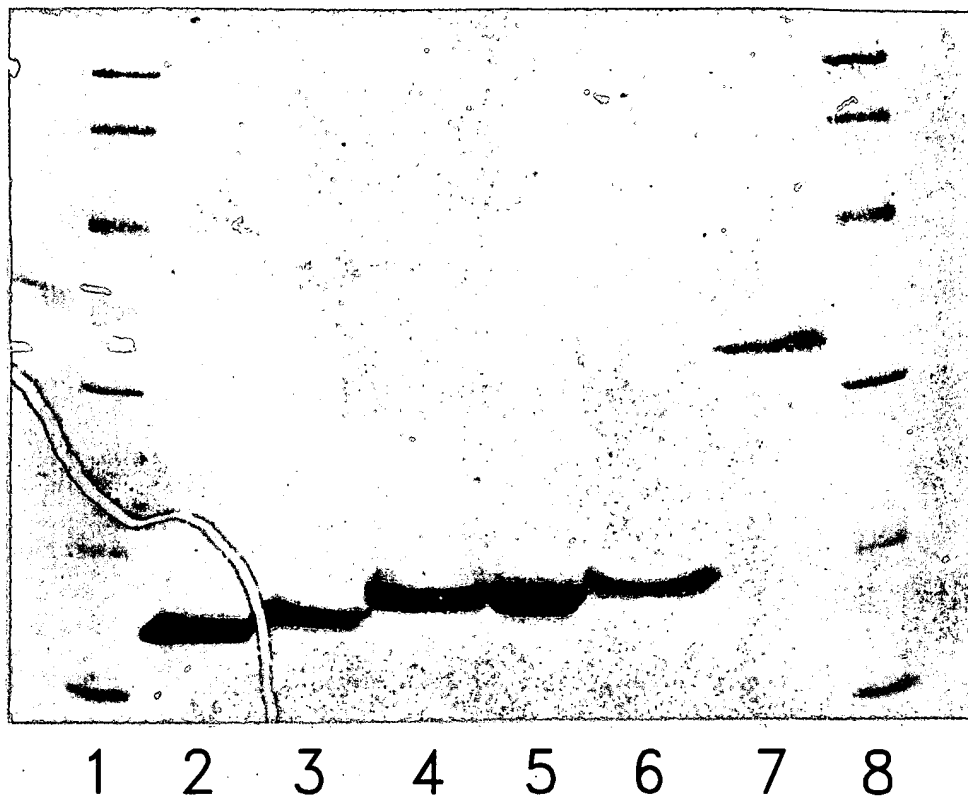
8- β -GALACTOSIDASE OF E. COLI(40 μ l OF THE CULTURAL MEDIUM)

FIG. 11



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ELECTROPHOREGRAM OF THE PURIFIED PRODUCTS:

1-MOLECULAR WEIGHT KIT (94 000,67 000,43 000,30 000
20 100, 14 4000)

2-haFGF 134

3-haFGF 140

4-haFGF 146

5-IFN α 2B

6-haFGF 155

7-MAP

8-MOLECULAR WEIGHT KIT

FIG. 12